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MiR-27b-3p targeting BDNF inhibits TrkB / CREB signaling pathway and improves IL-1 β induced chondrocytic inflammation

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*Running title:*Ruan et al,miR-27b-3p ATTENUATES CHONDROCYTE APOPTOSIS AND INFLAMMATION

KEYWORDS: Knee Osteoarthrit, miR-27b-3p, TrkB/CREB, pathway, BDNF

Abstract

Knee Osteoarthritis (KOA) is a chronic disease characterized by progressive disability and joint pain. Meniscus chondrocytes apoptosis is the main cause of reduced chondrocyte number and self-repair function. The purpose of this study was to investigate the role of miR-27b-3p in KOA.In this study, we found that the expression of miR-27b-3p was downregulated in cultured IL-1β treated chondrocyte and cartilage tissues in KOA. KOA overexpression evidently reduced IL-1β induced chondrocyte apoptosis and caspase-3 and caspase-9 expression.The upregulated iNOS and COX-2 mRNA and proteins expression was also inhibited by miR-27b-3p mimics. The expression of nitric oxide, PGE2, TNF-α and IL-6 was also inhibited by miR-27b-3p mimics. The target gene of miR-27b-3p was confirmed to be BDNF. TrkB/CREB pathway was proved to be the downstream pathway of miR-27b-3p/BDNF axis.The apoptotic cell percentage and nitric oxide, PGE2, TNF-α and IL-6 expression was induced by BDNF+IL-1β. This induction was inhibited by miR-27b-3p mimics. The cartilage tissues stained with safranin O results showed

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miR-27b-3p greatly decreased KOA induced cartilage degradation. The expression of BDNF 、 TrkB and p-CREB was inhibited by len-miR-27b-3p. MiR-27b-3p also reduced the expression of TNF-α 、 IL-6 and Bax, and increased Bcl-2 expression. These results indicated miR-27b-3p could applied to inhibit the development of KOA and miR-27b-3p/BDNF/TrkB/CREB pathway could serve as novel treatment target to handle KOA.

Introduction

Meniscus is the fibrous cartilage plate in the knee joint,the inside is a “C”shape and the outside is an “O”shape.The outer edge of the meniscus is thick, the inner edge is thin and concave, which is composed of collagen fibers, is an important part of the structure and function of the knee joint.It has the properties of relieving vibration, lubricating joints, reducing friction, increasing stress area, conducting load and maintaining the stability of knee joint(1).Trauma or natural aging can make meniscus appear different degrees of deformation, and then cause surrounding tissue edema、proliferation、make the knee joint lose normal function and stability, accelerate the degeneration of articular cartilage, and further lead to the occurrence of Knee Osteoarthritis (KOA).

Knee Osteoarthritis (KOA) is a widely spread disease that characterized by articular cartilage degradation,subchondral bone sclerosis,hyperostosis, osteochondral vessel formation and articular inflammatory reaction. KOA is a chronic progressive disease and a leading cause of disability (2). Patients with KOA suffer from joint pain, swelling and decreased living quality (3). More than 10% of adults over 60 have KOA, and the incidence is increasing with age and Body Mass Index (BMI) (4). However, there are no effective treatment therapies to reverse this process of KOA in clinic. Thus,clarifying the mechanism of KOA is critical to develop effective methods to treat KOA.

MicroRNAs are a cluster of non-coding RNAs with 18-15 neucleotides in length. They could be bind to the 3’ untranslated region of the target gene mRNA (5, 6). This bind could induce the degradation of mRNA or inhibit the translation of target gene mRNA (7,8). MiRNAs have widely proved to regulate cellular behavior in multi diseases, like cancer, spinal cord injury, hepatitis etc (7, 9). MiRNAs could regulate

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the process of KOA. MiR-140 is expressed in chondrocytes and regulate cartilage development (10).MiR-26a could regulate KOA progression via targeting FUT4/NF-κB pathway (11). MiR-489-3p has been reported to regulate tumorigenesis, bladder cancer cell migration and proliferation and prostate cancer progression (12-14). However, the role of miR-27b-3p in KOA has not been clarified.

The level of IL-1β is increasing in the cartilage tissue in KOA patients (15, 16). IL-1β is regarded as one of the two inflammatory factors in KOA onset and development (15). The increasing of other cytokines, like IL-6, could be induced by IL-1β. The catabolism is also induced by IL-1β, which results in cartilage degradation. Therefore, inhibiting the role of IL-1β is critical to reduce the process of KOA (17). Chondrocytes is the main cell type in cartilage. The main function of chondrocytes is to maintain the metabolism balance of cartilage.They can secrete collagen and proteoglycan to maintain the integrity and function of cartilage. However, IL-1β could also induce chondrocytes apoptosis to decrease the self-repair ability of cartilage (18). Thus, inhibiting chondrocyte apoptosis is of great meaning to treat KOA.

TrkB/CREB pathway is important in many bioprocess, like inflammation response, cell injury response and apoptosis (19). In KOA, the TrkB/CREB pathway is activated by IL-1β (20). Inhibiting activation of TrkB/CREB could decrease IL-1β induced chondrocyte apoptosis and showed beneficial effect on KOA (20). Evidence has showed brain-derived neurotrophic factor (BDNF) is upregulated in the joint tissues of KOA patients (21,22). BDNF is an extracellular cytokine that could actively TrkB/CREB pathway by binding to its cell membrane receptor, NTR. Thus, inhibiting BDNF could be an efficient way to inhibit the development of KOA via reducing TrkB/CREB activation.

In this study, we aimed to clarify the role of miR-489-3p in KOA in both in vitro and in vivo. The target gene of miR-27b-3p was confirmed to be BDNF and miR-27b-3p could regulate chondrocyte apoptosis via BNDF/TrkB/CREB pathway. This study could provide novel treatment target for KOA.

Methods

Animal and Ethic statement

A total of 96 male SD rats （250±0.05)g were obtained from Yan'an University

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Laboratory Animal Technology Co., Ltd. The rats were randomly separated into four groups: Control, KOA, KOA+Len-NC and KOA+Len-miR-27b-3p groups. Each group had 24 rats for RT-qPCR, histological detection, western blotting and ELISA. The rats were housed in 12 h light/dark cycles. All the procedures were approved by the ethics committee of Yan'an University.

Rat KOA Model

To detect the role of miR-27b-3p in KOA, rat KOA model was established as previously reported . The KOA rat model was established by transection of lateral meniscus of right posterior limb lateral meniscus cutting off.Rats were anesthetized by intraperitoneal injection of pentobarbital sodium, the skin of the right posterior limb lateral meniscus cut off. A para patellar incision was made, the patella was dislocated. Then, the lateral meniscus was cut. Then, the incision was sutured. The lentivirus overexpressing miR-27b-3p (1×10^9 units in 20 μL, twice a week for 7 weeks, n=24) was injected into the space of keen joint. After 8 weeks, rats were sacrificed through carbon dioxide and joint tissue samples were collected.

Chondrocyte Culture

Human chondrocytes (CH8) was obtained from Cavens (Changzhou, China). The cells were maintained with DMEM adding FBS (10%) and penicillin/streptomycin (1%). The cells were kept in CO_2 (5%) and 37 ℃ condition. The cell culture medium was changed every day. IL-1β (10 ng/mL, Sigma, USA) was applied to induce KOA in vitro. BDNF (10 ng/mL, Sigma, USA) was applied to treat cultured chondrocytes.

RT-qPCR

To detect the expression of BDNF, iNOS, COX-2 mRNA and miR-27b-3p in cultured chondrocytes and the expression of miR-27b-3p in cartilage tissue, the RT-qPCR was conducted as previously reported. The cell samples and cartilage tissues were harvested and the total RNA was extracted with TRIzol kit (Invitrogen,

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USA). Nanodrop 2000 was applied to determine the RNA concentration of samples. The cDNA of miR-489-3p was synthesized with ReverAid First Strand cDNA Synthesis (Thermo, USA). The cDNA of BDNF, iNOS and COX-2 mRNA was obtained with Primescript RT Master Mix (Takara, Japan). MiScript SYBR® Green PCR Kit (Thermo, USA) was applied to conduct RT-qPCR assay. GAPDH mRNA was applied as internal reference of BDNF, iNOS and COX-2 mRNA, while U6 was set as internal reference of miR-27b-3p. The relative expression of these genes was calculated with $2^{-\Delta\Delta Ct}$ method. The primers were shown in Table 1.

Western Blotting

The protein expression of caspase-3、caspase-9、iNOS、COX-2、BDNF、TrkB,CREB、p-CREB in cultured chondrocytes and the protein expression of BDNF、p-TrkB、p-CREB in cartilage tissues was detected with Western blotting. The total proteins in cells and tissues were harvested with RIPA buffer (Beyotime, China). Nanodrop 2000 was applied to determine the protein concentration of samples. Then, proteins in samples were separated by electrophoresis in SDS-PAGE. Then, the proteins were transferred onto PVDF membranes. The PVDF membranes were blocked with non-fat milk (5%). Then, the primary antibodies were applied to label target proteins. Then, the secondary antibody (goat-rabbit IgG-HRP 1:1000) was applied to incubate PVDF membranes. Then, ELC kit (Beyotime, China) was applied to show the blots. GAPDH was set as an internal reference. Antibodies were purchased from Abcam, UK.

ELISA Assay

The concentration of nitric oxide, PGE2, TNF-α and IL-6 in cultured chondrocytes, and the concentration of Bax, Bcl-2, TNF-α and IL-6 in cartilage tissues were detected with ELISA assay. Briefly, the samples were prepared according to the manufacturer's instruction. The ELISA kits were purchased from R&D, USA.

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Oligonucleotide Transfection

To detect the role of miR-27b-3p in KOA, the miR-27b-3p mimics and NC were transfected into cultured chondrocytes with the help of Lipofectamine2000 (Invitrogen, USA). These oligonucleotides all obtained from GenePharma, China. The cultured chondrocytes were seeded into culture plate at a density of 1×10^6 / mL. The transfection was conducted when the confluence reached 80%. 24 h after transfection, the cultured medium was replaced with fresh culture medium.

Flow Cytometry Assay

To demonstrate the role of miR-27b-3p on chondrocyte apoptosis, flow cytometry assay was conducted with FITC-AnnexinV apoptosis detection kit (Beyotime, China). The chondrocytes were harvested and rinsed with cold PBS. The chondrocytes were labeled with FITC-AnnexinV for 4 min on ice. The cells were rinsed with coupling solution. Then, PI was applied to incubate these cells. Then, apoptosis cells were detected with Fortessa flow-cytometer (BD Biosciences, US). The acquired data were analyzed with FlowJo software.

Dual-luciferase reporter assay

To clarify whether BDNF is the target gene of miR-27b-3p, dual-luciferase reporter assay was conducted. The binding sites of miR-27b-3p on BDNF mRNA were predicted by TargetScan database. The BDNF mRNA 3'UTR was cloned into p-MIR-GLO reporter assay (Promega, USA). The reconstructed plasmid was named BDNF-WT. In the same way, the BDNF-MUT plasmid with mutant binding sites was reconstructed. The cultured chondrocytes were co-transfected with miR-27b-3p mimics/NC and BDNF-WT/BDNF/MUT with lipofectamine2000. 48 h after transfection, the relative luciferase activities were detected by dual-luciferase reporter assay system (Promega, USA).

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Histological analysis

The cartilage tissues were harvested and fixed with paraformaldehyde (4%). The fixed tissues were embedded into paraffin and sliced (5 μm). The cartilage tissues were stained with safranin O staining. The pictures were obtained with the microscope (Nikon, Japan).

Statistical analysis

All data were recorded and analyzed with GraphPad Prism 6.0. The recorded data were presented as mean ± SD. The differences between more than three groups were analyzed by one-way ANOVA, followed by Turkey's multiple comparison test. $P<0.05$ was considered as statistically significant.

Results

MiR-27b-3p inhibition of IL-1β induced chondrocyte inflammation CH8 chondrocyte apoptosis

To further clarify the role of miR-27b-3p in KOA, our experiment transfected IL-1β treated chondrocytes with mir-27b-3p mimics.The expression of miR-27b-3p was greatly inhibited in IL-1β group compared with that in control group. This inhibition effect of IL-1β was totally reversed after miR-27b-3p mimics transfection (Fig.1A). IL-1β-treated chondrocytes can cause chondrocyte inflammation, the possible mechanism is that IL-1β can induce chondrocyte apoptosis.while miR-27b-3p mimics reduced the percentage of apoptotic cells (Fig.1B). IL-1β also induced the expression of caspase-3 and caspase-9, while miR-27b-3p greatly inhibited the role of IL-1β (Fig.1C). These results indicated that miR-27b-3p has a protective effect on chondrocyte apoptosis induced by IL-1β.

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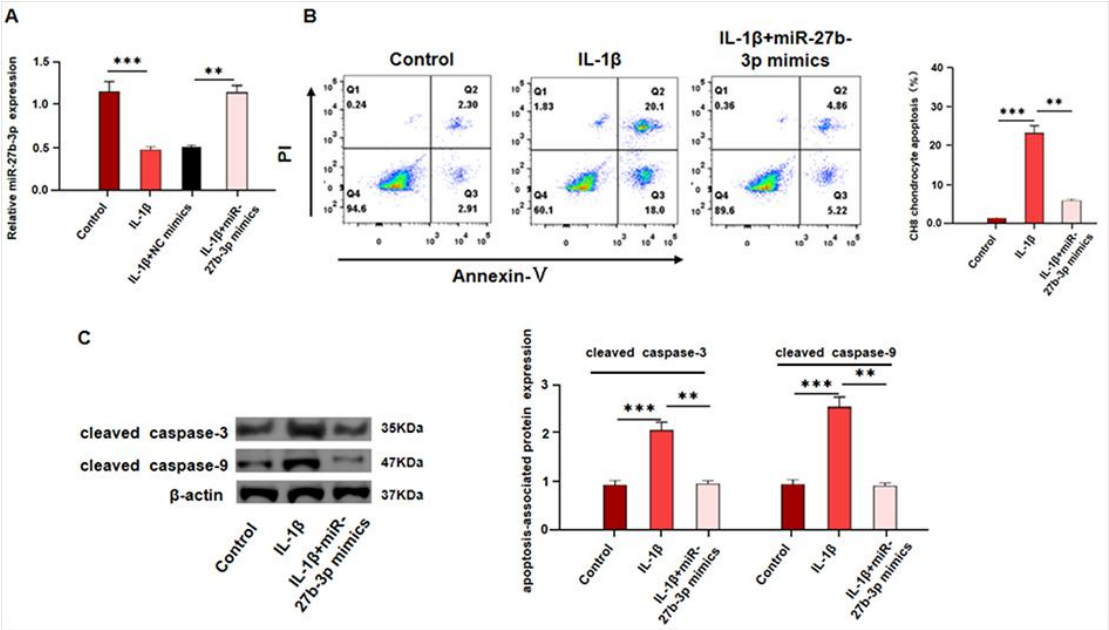

Figure 1. MiR-27b-3p inhibits IL-1β induced chondrocyte apoptosis.

(A) .The expression of miR-27b-3p detected by RT-qPCR. (B). The apoptotic cells detected by FITC-Annexin V flow cytometry assay. (C) .The expression of caspase-3 and caspase-9 detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

MiR-27b-3p can reduce the expression of inflammatory factors in IL-1β treated chondrocytes

IL-1β induced chondrocytes have inflammatory reaction and apoptosis, is there a certain relationship between them? Do miR-27b-3p play an anti-apoptotic role by inhibiting inflammatory response?To verify this assumption,we detected the expression of iNOS and COX-2 mRNA expression with RT-qPCR. IL-1β could be greatly induced iNOS and COX-2 mRNA expression, while miR-27b-3p evidently reduced their expression (Fig.2A). Similarly, miR-27b-3p could also reduce IL-1β upregulated iNOS and COX-2 protein expression (Fig.2B). The expression of nitric oxide, PGE2, TNF-α and IL-6 was also promoted by IL-1β treatment, and this promotion was greatly inhibited by miR-27b-3p mimics transfection (Fig.2C-F).

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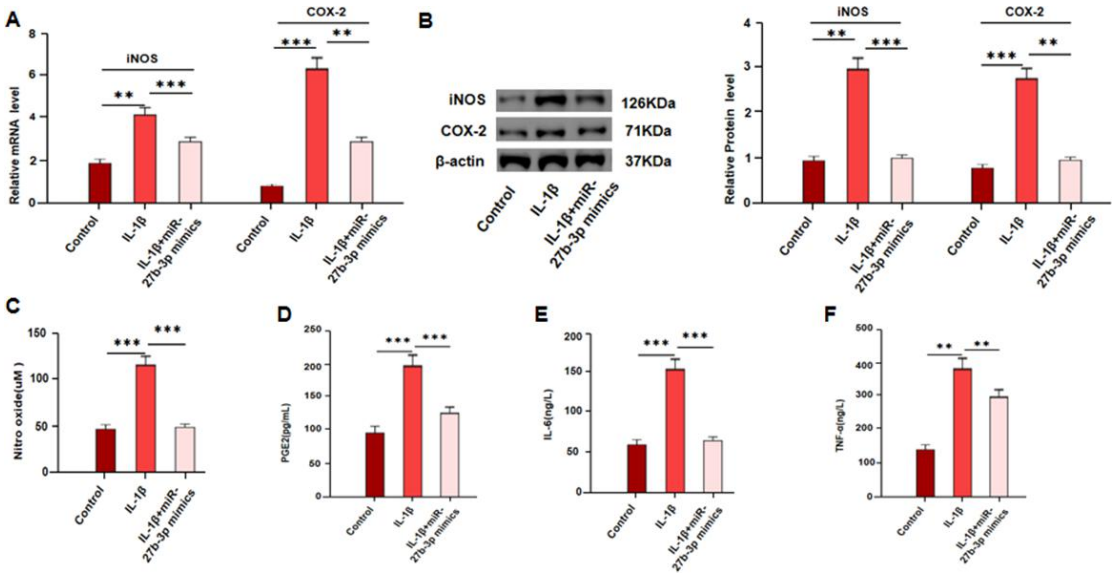

Figure 2.MiR-27b-3p inhibits the inflammatory response by reducing the expression of inflammatory factors in IL-1β treated chondrocytes.

The expression of iNOS and COX-2 mRNA detected by RT-qPCR (A). and Western blotting (B). The expression of nitric oxide (C)、PGE2 (D)、TNF-α (E) and IL-6 (F) detected by ELISA. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p$ <0.05).

Bioinformatics analysis of BDNF is the target relationship of miR-27b-3p

To further clarify the mechanism of action of MIR-27b-3p in KOA,the target gene of miR-27b-3p was predicted in Targetscan database, and BDNF was predicted to be the target gene of miR-27b-3p. The predicted binding sites were showed (Fig.3A). Dual-luciferase reporter assay showed the relative luciferase activity was greatly reduced in BDNF-WT+miR-27b-3p mimics group compared with the other three groups (Fig.3B).Then, the expression of BDNF mRNA was detected after miR-27b-3p mimics treatment. IL-1β greatly upregulated the expression of BDNF mRNA, while miR-27b-3p evidently inhibited the role of IL-1β on BDNF mRNA expression (Fig.3C). Similarly, the expression of BDNF protein was also promoted by IL-1β and inhibited by miR-27b-3p (Fig.3D). These results indicated BDNF was the target gene of miR-27b-3p .

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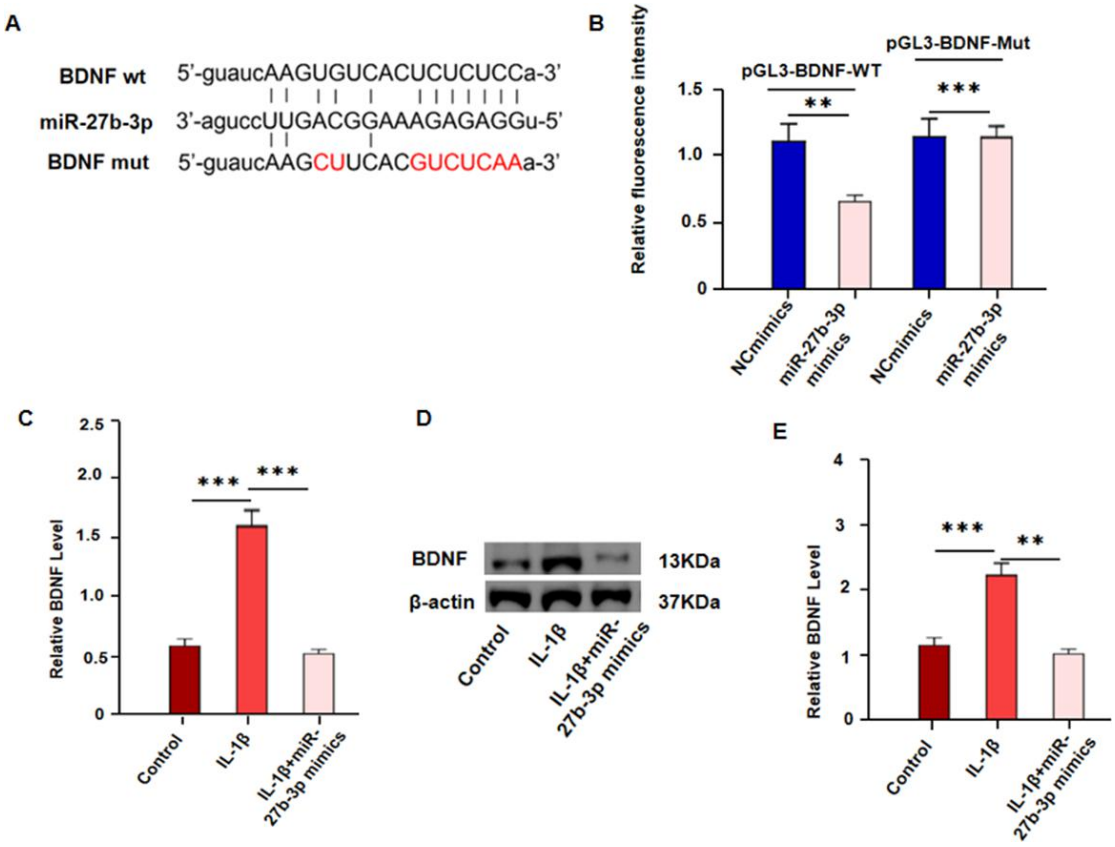

Figure 3. Target relationship analysis between BDNF and miR-27b-3p.

(A) .BDNF mRNA and miR-27b-3p binding sites predicted by Targetscan database. (B).The dual-luciferase reporter assay. (C) .The BDNF mRNA detected by RT-qPCR. (D). The expression of BNDF protein detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

MiR-27b-3p targeted BDNF regulate downstream TrkB/CREB pathways

To further clarify the mechanism of miR-27b-3p on KOA, we predicted the downstream pathway of miR-27b-3p/BDNF. Previous studies have shown that TrkB/CREB is the downstream pathway of miR-27b-3p /BDNF(22). This study further verifies this conclusion,we detected the expression of TrkB、p-TrkB、CREB and p-CREB in cultured chondrocytes. The expression of p-TrkB and p-CREB was greatly induced after IL-1β treatment, while their expression was inhibited by miR-27b-3p mimics (Fig.4 A). These results indicated miR-27b-3p could inhibit the TrkB/CREB pathway that triggered by IL-1β. To further confirm the regulative role of miR-27b-3p on BDNF triggered TrkB/CREB pathway, BDNF was applied to cultured chondrocytes. Unregulated expression of p-TrkB and p-CREB that induced by IL-1β

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was further increased after adding BDNF. Unregulated expression of p-TrkB and p-CREB that performed by IL-1β+BDNF was inhibited by miR-27b-3p mimics treatment (Fig.4 B). These results indicated miR-27b-3p could target BDNF to regulate TrkB/CREB pathway.

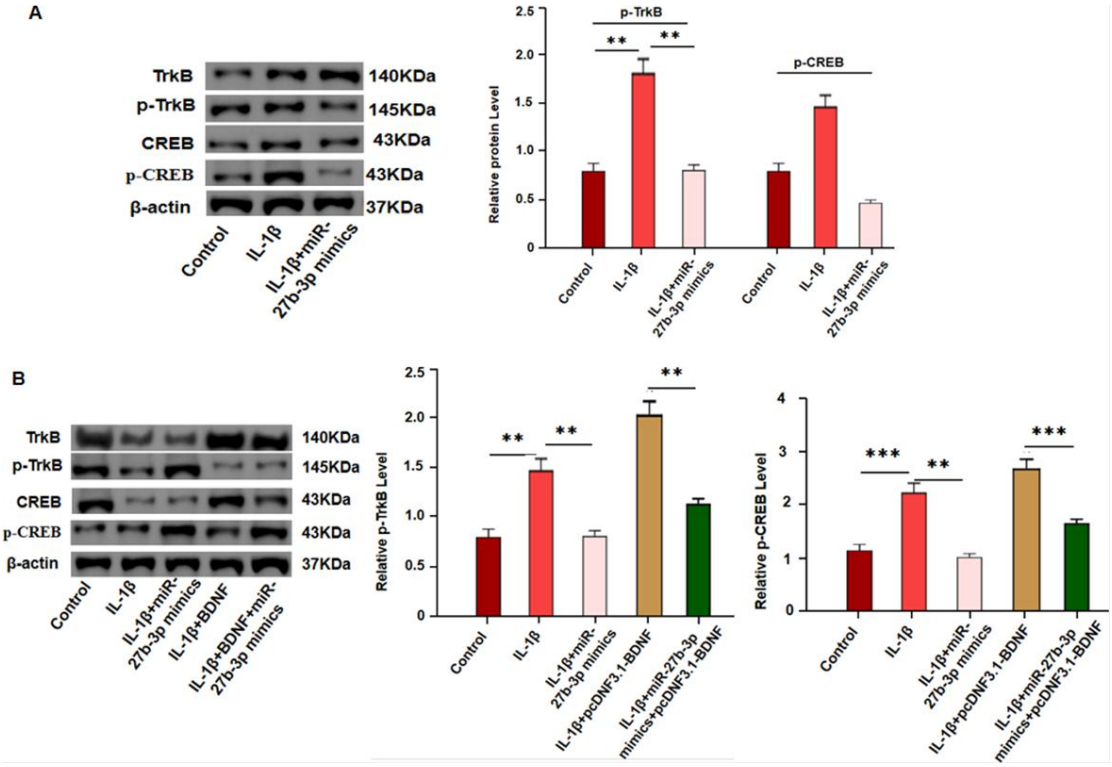

Figure 4. TrkB/CREB is the downstream pathway of miR-27b-3p targeted BDNF.

(A, B) .The expression of TrkB、p-TrkB、CREB and p-CREB in cultured chondrocytes detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

MiR-27b-3p inhibits IL-1β induced chondrocyte apoptosis and inflammatory factor secretion via targeting BDNF/TrkB/CREB pathway

To confirm whether the anti-apoptosis role of miR-27b-3p on IL-1β treated chondrocytes was via BDNF/TrkB/CREB pathway, the percentage of apoptotic cells was detected after overexpressing BDNF and miR-27b-3p.MiR-27b-3p reduced the percentage of apoptotic cells that promoted by IL-1β. IL-1β+BDNF further increased the percentage of apoptotic cells. The effect of IL-1β+BDNF was inhibited by miR-27b-3p(Fig.5A). These results indicated miR-27b-3p could inhibit IL-1β and BDNF induced apoptosis. The inflammatory factors, nitric oxide, PGE2, TNF-α and

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IL-6, were greatly promoted after IL-1β+BDNF treatment. The application of miR-27b-3p evidently inhibited the expression of these inflammatory factors that promoted by IL-1β+BDNF (Fig.5B-E). These results indicated miR-27b-3p could inhibit IL-1β induced chondrocyte apoptosis and inflammatory factor secretion via targeting BDNF/TrkB/CREB pathway.

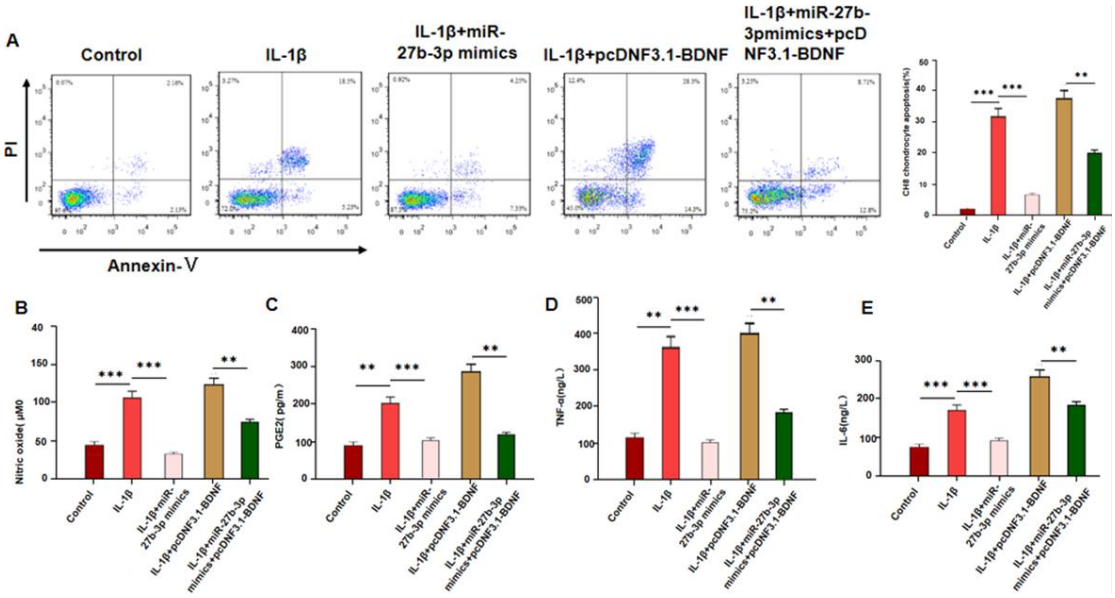

Figure 5. MiR-27b-3p inhibits IL-1β induced chondrocyte apoptosis and inflammatory factor secretion via targeting BDNF/TrkB/CREB pathway.

(A) .The apoptotic cells detected by FITC-AnnexinV flow cytometry assay. The expression of Nitric oxide (B)、PGE2 (C)、TNF-α (D) and IL-6 (E) detected by ELISA. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

In vivo experiments have shown that MiR-27b-3p reduces the degradation of extracellular matrix by inhibiting the expression of inflammatory factors, protects cartilage tissue, and relieves KOA pain

The above in vitro experiments prove that MiR-27b-3p can reduce KOA pain by inhibiting chondrocyte apoptosis and inflammation. In order to elaborate on this function of MiR-27b-3p, we further verify it through in vivo experiments. We established the rat model of KOA by knee meniscus dissection and injected miR-27b-3p mimics into the knee lumen to overexpress miR-27b-3p in the cartilage. In KOA model group, the expression of miR-27b-3p was evidently inhibited compared with that in control group. The application of Len-miR-27b-3p evidently increased the expression of miR-27b-3p compared with that in Len-NC group

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(Fig.6A). These results indicated miR-27b-3p was decreased in KOA cartilage tissues and Len-miR-27b-3p could increase miR-27b-3p expression of cartilage tissues. Then, the cartilage tissues were harvested and stained with Safranin O/Fast green. The cartilage in control group had large red stain area, the red area was greatly reduced in KOA model and KOA+Len-NC group. The application of miR-27b-3p evidently increased the red area compared with that in KOA+Len-NC group (Fig.6B). The expression of BDNF、 p-TrkB and p-CREB in cartilage tissues were greatly inhibited in KOA and KOA+Len-NC groups compared with that in control group. The application of Len-miR-27b-3p evidently decreased their expression (Fig.6C). Len-miR-27b-3p decreased Bax expression and promoted Bcl-2 expression that dysregulated in KOA and KOA+Len-NC group (Fig.6D、Fig.6E). The expression of TNF-α and IL-6 was greatly upregulated in KOA and KOA-Len-NC groups, and this upregulation was inhibited by Len-miR-27b-3p (Fig.6F、Fig.6G). These data showed miR-27b-3p protected by cartilage from degradation and inhibited inflammatory factors expression in vivo，to provide a good living environment for chondrocytes.

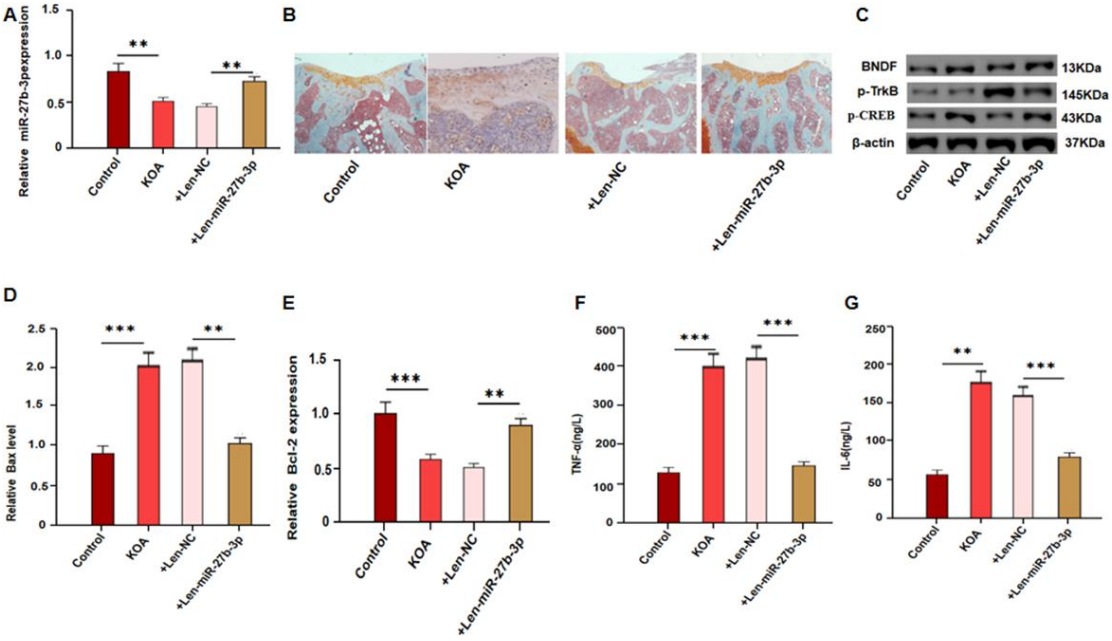

Figure 6. ***MiR-27b-3p reduces the degradation of extracellular matrix by inhibiting the expression of inflammatory factors, protects cartilage tissue, and relieves KOA pain.***

(A) .The expression of miR-27b-3p detected by RT-qPCR. (B). The cartilage tissues stained with safranin O. (C). The expression of BNDF, p-TrkB and p-CREB was

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detected by Western blotting. The expression of Bax (D)、Bcl-2 (E)、TNF-α (F) and IL-6 (G) in cartilage tissues. (n=10， ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

Discussion

The main pathological feature of KOA is degeneration and destruction of articular cartilage,because the cartilage cell lacks the nerve, the blood vessel and the lymph supply, as well as its own congenital differentiation and the migration ability is poor,the chondrocytes themselves have little ability to repair themselves,therefore, the current treatment of degenerative KOA cartilage is a major medical problem, how to repair the damaged articular cartilage and maintain its function is the treatment goal of osteoarthritis(23,24).

KOA is the leading disease that inducing disability in elderly people. However, there are no effective clinical treatment therapies to reverse the process of KOA(25, 26). This could be the result of the still uncovered molecular mechanism. Thus, clarifying the mechanism and developing novel treatment target are of great importance.

In recent years, miRNAs have been proved to be involved in the development of KOA (11, 26). Several miRNAs have been showed to regulate the pathogenesis of KOA (27-29). In this study, we confirmed miR-27b-3p was downregulated in IL-1β treated chondrocytes. Overexpressing miR-27b-3p inhibited IL-1β induced chondrocyte apoptosis and inflammatory factors secretion. Further, the target gene of miR-27b-3p was predicted to be BDNF. This prediction was proved by dual-luciferase reporter assay and BDNF mRNA and protein expression detection after miR-27b-3p overexpression. In in vivo study, miR-27b-3p overexpression inhibited cartilage degradation and inflammatory factors secretion. Thus, miR-27b-3p could exert positive role in KOA.

As chondrocyte is the only type cell in normal cartilage tissues, the apoptosis of chondrocyte in KOA greatly reduces the self-repair function of cartilage tissues. Inhibiting chondrocyte apoptosis will reduce the degradation of extracellular matrix (ECM) and could be a novel treatment aspect (30). In this study, miR-27b-3p could reduce chondrocyte apoptosis via targeting BDNF. BDNF is upregulated in the joint

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tissue in KOA, and this upregulation could induce KOA related pain (31). As an extracellular cytokine, BDNF could induce the activation of an intracellular pathway, like TrkB/CREB (32). Activation of TrkB/CREB pathway by IL-1β could induce chondrocyte apoptosis. Inhibiting TrkB/CREB pathway exerts the beneficial role in KOA (33,34,35). In this study, the TrkB/CREB pathway was inhibited by miR-27b-3p mimics. This pathway was further activated by IL-1β+BDNF. The role of IL-1β+BDNF was inhibited miR-27b-3p mimics. Our data showed miR-27b-3p could target BDNF to inhibit TrkB/CREB pathway activation and chondrocyte apoptosis(36).

The inflammatory reaction is induced in KOA. IL-6、nitric oxide、PGE2、COX-2、iNOS and TNF-α were upregulated in both cartilage tissues and IL-1β treated chondrocytes in KOA. In this study, miR-27b-3p could evidently reduce these inflammatory factors expression. These data showed miR-27b-3p could inhibit the inflammatory reaction in KOA. MiR-27b-3p might inhibit the chondrocytes apoptosis via inhibiting the inflammatory reaction.

In conclusion, we found miR-27b-3p was downregulated in KOA, and upregulating miR-27b-3p could protect chondrocyte from IL-1β induced cell apoptosis. MiR-27b-3p could protect chondrocytes against apoptosis via regulating BDNF/TrkB/CREB pathway.Finding a safe and effective KOA treatment method has become a hot research topic. This study, clarified a novel mechanism of KOA and proved a new treatment target for KOA.To sum up, miR-27b-3p can promote the repair of KOA cartilage through various mechanisms, and more comprehensive and in-depth research is still needed in the future to further verify the effectiveness and safety of miR-27b-3p treatment KOA.

Declarations

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

The analyzed data sets generated during the study are available from the

corresponding author on reasonable request.

Authors' contributions

CLR design the research and revised the article; RJC and MW drafted the manuscript; LJW and YY collected the data and analyzed the data. XJL and HXL did the statistical analysis.

Ethical approval and consent to participate

This study was approved by the ethics committee of Yan'an University and in accordance with Tenth People's Hospital Affiliated to Tongji University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table 1. mRNA sequence

	Sense	Anti-sense
miR-27b-3p	GTGACATCACATATACGG	GTGACATCACATATACGG
BDNF	GGCTGACACTTTTGAGCACGTC	CTCCAAAGGCACTTGACTGCTG
iNOS	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
COX-2	GCGACATACTCAAGCAGGAGCA	GCGACATACTCAAGCAGGAGCA
U6	AGTAAGCCCTTGCTGTCAGTG	CCTGGGTCTGATAATGCTGGG
GAPDH	AATGGACAACTGGTCGTGGAC	CCCTCCAGGGGATCTGTTTG

Figure Legends

Figure 1. MiR-27b-3p inhibits IL-1β induced chondrocyte apoptosis.

(A). The expression of miR-27b-3p detected by RT-qPCR. (B) .The apoptotic cells detected by FITC-Annexin V flow cytometry assay. (C) .The expression of caspase-3 and caspase-9 detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$)

Figure 2. MiR-27b-3p reduces the expression of inflammatory factors in IL-1β treated chondrocytes.

(A). The expression of iNOS and COX-2 mRNA detected by RT-qPCR (A) and Western blotting (B). The expression of nitric oxide (C)、PGE2 (D)、TNF-α (E) and IL-6 (F) detected by ELISA. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

Figure 3. BDNF is the target gene of miR-27b-3p.

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(A).The predicted binding sites of miR-27b-3p on BDNF mRNA. (B).The dual-luciferase reporter assay. (C) .The BDNF mRNA detected by RT-qPCR. (D) .The expression of BNDF protein detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

Figure 4. JAK/STAT3 is the downstream pathway of miR-27b-3p targeted BDNF.

(A, B) .The expression of JAK, p-JAK, STAT3 and p-STAT3 in cultured chondrocytes detected by Western blotting. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

Figure 5. MiR-27b-3p inhibits IL-1β induced chondrocyte apoptosis and inflammatory factor secretion via targeting BDNF/JAK/STAT3 pathway.

(A) .The apoptotic cells detected by FITC-AnnexinV flow cytometry assay. The expression of Nitric oxide (B),.PGE2 (C).TNF-α (D) and IL-6 (E) detected by ELISA. (n=8, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).

Figure 6. MiR-27b-3p protects cartilage from degradation and inhibits inflammatory factors expression in vivo.

(A) .The expression of miR-27b-3p detected by RT-qPCR. (B) .The cartilage tissues stained with safranin O. (C) .The expression of BNDF, p-JAK and p-STAT3 was detected by Western blotting. The expression of Bax (D). Bcl-2 (E)、TNF-α (F) and IL-6 (G).In cartilage tissues. (n=10,ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$).